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A new minimally invasive technique to show nerve ischaemia in diabetic neuropathy

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Abstract

Aims/hypothesis. Experimental studies have shown that abnormalities of nerve microcirculation are important factors in the pathogenesis of diabetic neuropathy but there have been few clinical studies. We have applied microlightguide spectrophotometry to measure intravascular oxygen saturation (HbO_2%) and blood flow in human sural nerve.
Methods. We studied ten patients with mild-moderate sensory motor diabetic neuropathy, nine patients without neuropathy and nine control subjects. We took 300 measurements of oxygen saturation under direct visual control through a 1.9 mm rigid endoscope over three regions of the nerve. Spectrophotometric measurements of nerve fluorescence were taken after an intravenous injection of sodium fluorescein and the rate of increase in nerve fluorescence (rise time) was used as an indicator of nerve blood flow.
Results. Nerve oxygen saturation was reduced in patients with neuropathy compared with control subjects (67.1 ± 2.2 % vs 76.7 ± 2.1 %, $p = 0.006$). Fluorescein rise time was prolonged in patients with neuropathy compared with the control group (48.5 ± 7.0 s vs 14.0 ± 3.1 s, $p = 0.001$) suggesting impaired nerve blood flow. There was a correlation between rise time, nerve oxygen saturation, glycaemic control and sural nerve sensory conduction velocity ($p < 0.01$).
Conclusion/interpretation. The combination of microlight-guide spectrophotometry and micro-endoscopy provides a valuable minimally invasive technique for clinical investigation of nerve microcirculation. We have shown reduced nerve oxygenation and impaired blood flow in diabetic neuropathy and these findings strongly support a central role of microvascular disease in the pathogenesis of diabetic neuropathy. [Diabetologia (1999) 42: 737–742]

Keywords Diabetic neuropathy, spectrophotometry, sural nerve, nerve blood flow, oxygen saturation.

Despite extensive research, the pathogenesis of diabetic neuropathy is far from clear. Most of the debates have focused on metabolic [1, 2] or vascular factors [3, 4] and the complex interactions between them [5, 6]. The role of microvascular disease in the pathogenesis of diabetic neuropathy has gained support from studies in experimental and human diabetic neuropathy [3]. In human sural nerve, oxygen tension was found to be reduced and the presence of arteriovenous shunting and impaired nerve blood flow shown [7, 8]. These findings have been reinforced by nerve biopsy studies, which have shown a correlation between the presence and the degree of microvascular abnormalities with the presence and severity of diabetic neuropathy [9–10].

In vivo studies involving human nerves have, however, been limited by the invasive nature of the mea-

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Abbreviations: HbO_2%, Intravascular oxygen saturation; MNSI, Michigan Neuropathy Screening Instrument; MDNS, Michigan Diabetic Neuropathy Score; BG, background retinopathy; PR, proliferative retinopathy; FAT, fluorescein appearance time; FRT, fluorescein appearance time.

surement techniques used. There is thus a need for new techniques, which allow a better understanding of the various pathophysiologic changes taking place in nerves and are suitable to use in clinical studies. These could also provide methods for direct assessment of the effect of therapeutic agents. We have developed a minimally invasive method for measurement of human sural nerve intravascular oxygen saturation (HbO_2%) and indices of blood flow. The measurements use the relatively new technique of microlightguide spectrophotometry to measure human sural nerve HbO_2% [11]. These were combined with spectrophotometric measurements of nerve fluorescence following a bolus dose of intravenous Sodium Fluorescein, to provide an index of nerve perfusion. We have used these to investigate the role of haemodynamic factors in the pathogenesis of diabetic neuropathy in subjects with and without peripheral diabetic neuropathy.

Subjects and methods

We studied ten subjects with mild to moderate sensory motor diabetic neuropathy, nine non-neuropathic diabetic subjects and nine healthy control subjects. Subjects with Type I (insulin-dependent) and subjects with Type II (non-insulin-dependent) diabetes mellitus were included. Informed consent was obtained from all subjects and the local ethics committee approved the study.

All subjects were screened for neuropathy using, the Michigan Neuropathy Screening Instrument (MNSI) and classified into the three groups using the Michigan Diabetic Neuropathy Score (MDNS). The MNSI and MDNS combined with nerve conduction studies provide a practical quantitative clinical and electrophysiological assessment for the diagnosis and staging of diabetic neuropathy and correlate well with other available methods for classification [12]. Therefore, all subjects underwent the following: 1) full history and clinical examination including MNSI and MDNS, 2) median and peroneal motor conduction velocities and median, sural and ulnar sensory conduction velocities at a skin surface temperature of 33 ± 1 °C using a Dantec 2000M electrophysiological system (Dantec, Bristol, UK), 3) ankle pressure index using a Doppler ultrasound stethoscope, model BF4A (Med Sonics, Mountain View, Calif., USA) following a method described previously [13], 4) vibration perception threshold over the great toe using the biothesiometer (Biomedical Instrument, Newbury, Ohio, USA), 5) fundoscopy through dilated pupils using Tropicamide 2%. Subjects were classified as having normal fundoscopy, background retinopathy (BG) or proliferative retinopathy (PR) with or without previous laser treatment.

Subjects were excluded from the study if they had any of the following: 1) history of significant cardiac or chest disease, 2) the presence of significant peripheral vascular disease with either absent foot pulses or ankle pressure index less than 1, 3) peripheral neuropathy due to causes other than diabetes, 4) taking vasoactive drugs or substances including anti-hypertensive drugs, 5) smokers.

Subjects were classified using MDNS as class 0 or no neuropathy if they had 0–1 abnormal nerves and a clinical score of 6 or less, class 1 or mild neuropathy if they had 2 abnormal nerves and a clinical score of 12 or less, class 2 or moderate neuropathy if they had 3–4 abnormal nerves and a clinical score of 29 or less and class 3 or severe neuropathy if they had 5 abnormal nerves and a clinical score of 46 or less.

Equipment. The Erlangen micro-lightguide spectrophotometer II (EMPHO II, Bodenseewerk Gerätetechnik, Überlingen, Germany) was used in this study. The equipment set-up has been described in detail elsewhere [14] and is only described briefly here. Light from a 75-W Xenon lamp is carried by a single fibre (diameter 250 μm) to the surface of the tissue. The reflected light is transferred by six fibres surrounding the transmitting fibre to a rotating interference filter disk. The light is monochromatized in a range between 502–628 nm, detected by a photomultiplier and the electrical signal digitised for processing by computer. The shape of the measured haemoglobin spectra is compared to reference haemoglobin spectra and HbO_2% calculated by an on-line program as described previously [11].

A rigid endoscope with an outer diameter of 1.9 mm (Storz, Tuttlingen, Germany, Type 28301B) was attached via a 80/20 beam splitter (Storz, Type R5199) to a monochrome video camera (Hitachi KP-M1). For these endoscopic measurements a separate 250 W Halogen light source (Storz Cold Light Fountain 485BF) was used at the lowest power setting, to illuminate the nerve. The advantage of this combination is that the exact site of measurement can be made visible. At the start of each study a 'white balance' calibration with a surface coated mirror was used to correct for differences in the spectral composition of the halogen light source.

Sural nerve intravascular oxygen saturation measurement (HbO_2%). All surgical procedures were carried out in the hospital outpatient day care centre by an experienced neurosurgeon who had no previous knowledge of the subject's clinical state.

To minimise the size of the incision needed to expose the nerve, the position of the sural nerve was mapped along the ankle a day before the procedure. Small current pulses were applied by 10 mm bipolar stimulating electrodes. The stimulus was reduced to threshold sensation and the electrode moved across the skin in steps to identify points of maximum sensitivity along the presumed course of the nerve.

A venflon catheter was inserted into the cephalic vein and subjects were laid on their sides with their right leg immobilised by means of a cradle. After infiltration of the skin by 2% plain Lignocaine, a 0.5–1 cm incision was made along the mapped site of the sural nerve.

The nerve was exposed using blunt dissection, taking care not to disturb the epineurial blood vessels. The mean HbO_2% value was calculated from 3 groups of 100 measurements taken at separate sites along the nerve, using the combination of the endoscope and micro-lightguide spectrophotometer. The endoscope was mounted on a micromanipulator and was guided into position using the image of the nerve displayed on the video monitor. The measurements were taken with the tip of the endoscope approximately 0.5 mm away from the surface of the nerve. After each series of measurements, the tip of the endoscope and the exposed nerve were washed with warm saline and dried. During the procedure, temperature measurements were made by placing a needle thermocouple (Cole-Parker Instruments, Chicago, Ill., USA) into the tissue alongside the nerve. The blood glucose of the diabetic subjects was checked to ensure that it was between 6 and 10 mmol/l throughout the procedure.

Measurement of indices of nerve blood flow. Nerve fluorescence was then measured over the central portion of the exposed nerve. The tip of the endoscope was placed 1 mm from

the surface and the patient given one millilitre per 20 kg body weight of 20% Sodium Fluorescein (Martindale Pharmaceutical, Romford, UK) as a bolus intravenously into the right cephalic vein. Fluorescein excitation was illicit using the Storz light source (maximum intensity setting) fitted with a blue barrier filter. Fluorescence within the nerve was measured for 5 min, during this time the position of the endoscope over the nerve was monitored via the camera and recorded on a Sony U-matic video recorder (Sony, Japan). Subjects in whom there were significant changes in the position of the nerve under the endoscope were excluded.

Nerve fluorescence was quantified directly from the uncorrected spectra by subtracting the baseline intensity values and integrating between 510 and 560 nm. We calculated the time to first appearance of fluorescence, the fluorescein appearance time (FAT) and the time between 10 and 90% maximum intensity, the Fluorescein rise time (FRT) of the fluorescein curve [15].

Statistics. Statistical analysis was carried out using SPSS/PC (SPSS, Chicago, Ill., USA). Data were analysed using Spearman's rank correlation.

Results

Table 1 shows the clinical characteristics of each group. Subjects in the two diabetic groups were slightly older than control subjects but there was no statistically significant difference in age between the three groups. There was no significant difference in glycaemic control or duration of diabetes between the two diabetic groups. There was no significant difference between the three groups in tissue temperature.

Sural nerve intravascular oxygen saturation (HbO_2%). The mean HbO_2% in subjects with diabetic neuropathy was reduced compared with the normal control subjects ($p = 0.006$). The mean HbO_2% in diabetic subjects with neuropathy, without neuropathy and control subjects was 67.1 ± 2.1%, 72.4 ± 1.6% and 76.7 ± 2.1%, respectively (Table 2). Figure 1 is a scatter plot showing the HbO_2% in each of these groups. The value of the mean HbO_2% in the diabetic subjects without neuropathy was not significantly different from the other two groups.

Fluorescein indices of nerve blood flow. The results of the indices of nerve blood flow are shown in Table 2. Fluorescein rise time was prolonged in subjects with diabetic neuropathy compared with healthy control subjects (48.5 ± 3.1 vs 14.0 ± 3.1 s, $p = 0.001$) (Fig. 2). In diabetic subjects without neuropathy FRT was longer than in control subjects but did not reach statistical significance (26.0 ± 7.2 s). A scatter plot showing the FRT values in each of the three groups is shown in Figure 3. There was no significant difference in FAT between diabetic subjects with, without neuropathy and control subjects (31.7 ± 4.0, 48.4 ± 6.0 and 36.1 ± 3.6 s, respectively).

Table 1. Clinical characteristics of study subjects

	Normal control	Diabetic with neuropathy	Diabetic without neuropathy
Number	9	10	9
Age (years)	50.8 ± 2.5	55.0 ± 3.1	56.5 ± 2.0
Male : Female	6:3	9:1	5:4
Duration (years)	–	20.4 ± 2.7	21.1 ± 5.4
Type I : II	–	7:3	7:2
HbA_1 (3.3–6.8%)	5.0 ± 0.15	10.4 ± 0.8	11.1 ± 0.9
Retinopathy	–	3PR, 3BG	1PR, 1BG
Neuropathy score	–	4 Mild, 6 Moderate	–

Results expressed as means ± SEM

Table 2. Results of sural nerve intravascular oxygen saturation HbO_2 (%), fluorescein appearance time and fluorescein rise time

	Normal control $n = 9$	Diabetic with neuropathy $n = 10$	Diabetic without neuropathy $n = 9$
HbO_2 (%)	76.7 ± 2.1 $n = 8$	67.0 ± 2.2[a] $n = 8$	72.3 ± 1.6 $n = 8$
Fluorescein appearance time (seconds)	36.1 ± 3.6 $n = 5$	31.7 ± 4.0 $n = 7$	48.4 ± 6.0 $n = 7$
Fluorescein rise time (seconds)	14.0 ± 3.1 $n = 6$	48.5 ± 7.0[a] $n = 7$	26.1 ± 7.2 $n = 5$

[a] $p < 0.01$.
Results expressed as means ± SEM

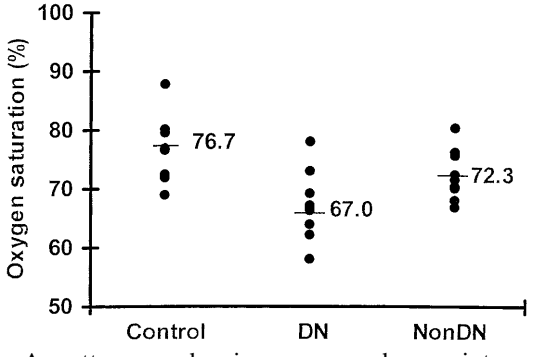

Fig. 1. A scattergram showing mean sural nerve intravascular oxygen saturation in the control group and in the diabetic subjects with (DN) and without neuropathy (NonDN)

Correlation between FRT, HbO_2% glycaemic control and electrophysiological measurement. There was a correlation between FRT and HbO_2%, HbA_1, sural nerve sensory conduction velocity and peroneal nerve motor conduction velocity ($r = -0.66$, 0.58, –0.57 and –0.47, respectively) (Table 3).

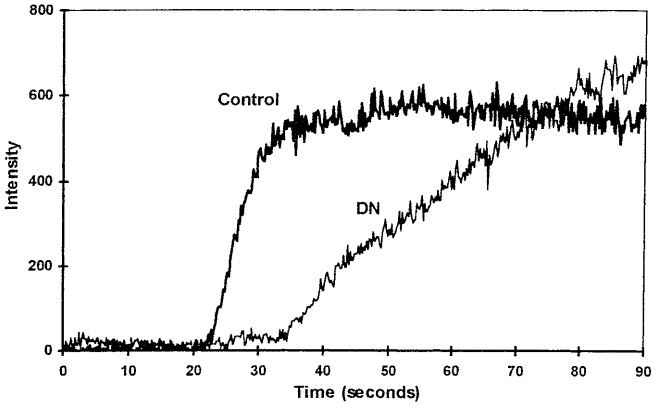

Fig. 2. Examples of nerve fluorescence curves of a control subject and a diabetic subject with neuropathy (DN)

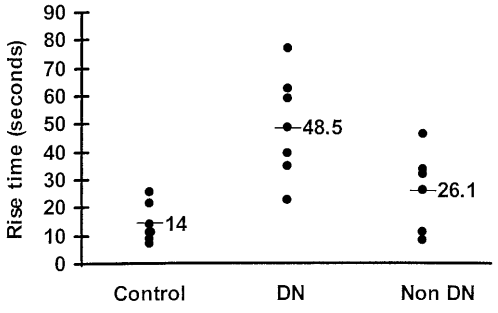

Fig. 3. A scattergram showing mean sural nerve fluorescein rise time in the control group and in the diabetic subjects with (DN) and without neuropathy (NonDN)

Table 3. Correlation between fluorescein rise time and sural nerve oxygen saturation, sural nerve sensory conduction velocity, peroneal nerve motor conduction velocity and HbA_1

Variable	*r*	*p* Value
Oxygen saturation	– 0.66	< 0.01
Sural nerve sensory conduction velocity	– 0.57	0.014
Peroneal nerve motor conduction velocity	– 0.47	< 0.05
HbA_1	0.58	0.01

2-tailed Spearman rank correlation

Discussion

We have applied the combination of microendoscopy and microlightguide spectrophotometry for the measurement of human sural nerve intravascular oxygen saturation and indices of nerve perfusion in diabetic neuropathy. The technique is minimally invasive requiring only a 1 cm incision compared with the 4–6 cm incisions used in previous studies. This was achieved by mapping the position of the nerve prior to the procedure and using microendoscopy to make the nerve visible and take measurements over its surface. Microelectrode polarography is the only other method that has been used to measure endoneurial oxygen tension in human sural nerve [7]. The technique is still widely used in experimental diabetic neuropathy studies [16, 17]. It involves repeated insertion of the microelectrode into the nerve body over several minutes. It is not known whether this causes local damage with corresponding changes in the measured variables.

We have shown that HbO_2% is reduced in subjects with diabetic neuropathy compared with normal control subjects. In a previous study from our group using microelectrode polarography it was shown that endoneurial oxygen tension was reduced in subjects with diabetic neuropathy [7]. In this report mean sural nerve oxygen tension in the neuropathy group and the control group were 39.7 and 52.1 mmHg, respectively which is equivalent to an oxygen saturation of 70.3 % and 80.5 %. The differences between the two techniques and the possible differences in the compartments measured, should, however, be taken into consideration when making direct comparison between the two studies. Our technique assumes that in the microcirculation there is an equilibrium between mixed arteriolar and venous intravascular oxygen and nerve fibre oxygen tension. Therefore, the intravascular oxygen saturation reflects the balance between nerve blood flow and the rate of oxygen extraction by nerve fibres. This is supported by the correlation between intravascular oxygen saturation and FRT in this study.

We found FRT to be longer in subjects with diabetic neuropathy compared with normal control subjects, suggesting impaired nerve perfusion and blood flow. We have previously used fluorescein angiography with video microscopy to measure indices of human sural nerve perfusion in diabetic neuropathy [8]. In this study we used the spectrophotometer to monitor nerve fluorescence as this is a more sensitive method, given the relatively low light intensity through the endoscope. We have also used the fluorescein rise (FRT) in addition to the fluorescein appearance time (FAT). Although FRT is influenced by cardiac output, it is directly related to local tissue blood flow and is a measurement of the blood velocity [15]. We have found no significant difference in FAT between the two groups. This is not surprising as FAT reflects the effect of vascular factors before the nerve, such as cardiac output and peripheral vascular resistance and represents mainly the transient time through large vessels.

Rats with experimental diabetic neuropathy have reduced nerve blood flow [16], which can be as low as 50 % of normal after 1 week of induction of diabetes [18]. Improvement of nerve blood flow following vasodilator treatment [19, 20] resulted in prevention or correction of neuropathy in those animals. In human diabetic neuropathy only a few studies have been carried out to assess nerve blood flow. In one the presence of arterio-venous shunting and impaired blood flow in patients with moderate to severe dia-

betic neuropathy was shown using fluorescein angiography [8]. There is also indirect evidence indicating impaired nerve blood flow in diabetic neuropathy. There is a pronounced reduction of exercise-induced conduction velocity increment in patients with diabetic neuropathy [21]. A correlation between peroneal motor conduction velocity and leg transcutaneous oxygen tension has been shown [22]. There is also an improvement of nerve conduction velocity after 3 months treatment with Lisinopril in subjects with diabetic neuropathy [23]. In this study we have shown reduced nerve perfusion in diabetic neuropathy and that it is associated with reduced intravascular HbO_2%. There has been one recent report of raised sural nerve blood flow in subjects with mild diabetic neuropathy using laser Doppler flowmetry [24]. In that study patients with diabetic neuropathy were, however, compared with patients with other forms of polyneuropathy and there was no statistically significant difference between all groups.

Impairment of nerve blood flow in diabetic neuropathy could be caused by different mechanisms. Many morphometric studies have shown structural changes in the endoneurial blood vessels in diabetic neuropathy. These include reduplication of the basement membrane [9], endothelial cells hyperplasia [25] which can lead to occlusion of capillaries [26], increased plugging of vessels by cell debris [27] and degeneration of pericytes [4]. In addition a number of haemorrheological abnormalities in diabetes may contribute to impaired blood flow. Increased blood viscosity [28], reduced erythrocyte deformability [29] and platelet abnormalities [30] have been documented in diabetes. Denervation of epineurial vessels in diabetic neuropathy [31] may also lead to reduced endoneurial blood flow and oxygen saturation as a result of increased arterio-venous shunting [8]. Furthermore, it has been suggested that increased vasa nervorum production of the vasoconstrictors, endothelin and angiotensin II and the reduction of vasodilatation by nitric oxide and prostaglandin I_2 in diabetic neuropathy might lead to further reduction of nerve blood flow [6].

Intensive insulin therapy or pancreatic transplantation [32, 33] have been shown to prevent or slow the progression of diabetic neuropathy. The exact mechanism as how this effect is exerted is, however, still not clear [34]. In this study we have shown that there is an association between FRT and HbO_2%, glycaemic control and nerve conduction velocity. This suggests that improved glycaemic control may have a direct effect on nerve blood flow with subsequent effect on nerve function.

The minimally invasive nature of this technique makes it suitable for repeated measurements on the same subject and is a method to monitor the effects of potentially useful therapeutic agents. There were very few adverse effects from the procedure. In the group we studied, there were two subjects who had transient nausea following fluorescein injection and two had a mild skin infection at the site of the incision.

In conclusion, the combination of microlight-guide spectrophotometry and micro-endoscopy provides a valuable minimally invasive technique for clinical investigation of microvascular oxygen and blood flow. We have found reduced nerve oxygenation and impaired blood flow in diabetic neuropathy as shown in previous studies. There is also a good correlation between nerve blood flow and nerve oxygenation, glycaemic control and electrophysiological measurements. These findings strongly support a central role of microvascular disease in the pathogenesis of diabetic neuropathy.

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